1983

Enteric infections in Stockton, California : with special reference to the genus Campylobacter : a thesis ...

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ENTERIC INFECTIONS IN STOCKTON, CALIFORNIA WITH SPECIAL REFERENCE TO THE GENUS CAMPYLOBACTER

A Thesis

Presented to

the Faculty of the Department of Biological Sciences

University of the Pacific

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

by

Tom E. Hathorn

May 1983

This dissertation, written and submitted by

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is approved for recommendation to the Committee on Graduate Studies, University of the Pacific

Dean of the School or Department Chairman:

Dissertation Committee:

Chairman

Dated May 5, 1983

ACKNOWLEDGEMENT

Sincere appreciation goes to Dr. F. M. Nahhas for his expertise, guidance, patience and willingness to give his time. Thanks go to Doug Smith for being generous with his time and for giving helpful suggestions for my computer programming. Thanks also go to Dennis Ferrero for reference information concerning enteric culturing in California and San Joaquin County in particular.

I would also like to express deep gratitude to Bill Mortola for his valuable tips in thesis preparation and for his helpful peer encouragement. Finally, much credit is due Laura O'Donnell for being my moral support, for balancing the academic aspects of my life, and for understanding the importance of my work.

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INTRODUCTION

Diarrheal disease is one of the most common maladies suffered by man. It may range in severity from the lesser forms of mild diarrhea, nausea and cramping to the most severe syndromes of watery and bloody stools, vomiting and high fever. The milder to intermediate forms are commonly known as enteritis, enteric fever and gastroenteritis; the most severe syndromes include the age-old diseases of Asiatic cholera, typhoid and certain forms of bacillary dysentery (shigellosis). Dozens of microorganisms are potential agents of such infections, especially the less severe forms. Among these agents are bacteria, viruses, yeasts and protozoa. The infections may be due to local damage inflicted by the organisms themselves, or caused by toxins produced locally or preformed prior to ingestion.

In spite of the fact that many microbes can cause enteritis, there are several reasons why microbiological techniques are only designed to search for a limited number of pathogens: the low potential for disease production of many microorganisms, the geographical distribution of certain pathogens, and the limitations imposed by time and expense. The low potential for disease production refers to the findings that certain microorganisms are opportunists, causing infections in patients who are debilitated, immunologically compromised or on prolonged antibiotic

treatment, whereas the true pathogens are nearly always associated with disease. Furthermore, the opportunists are found in the normal flora, and their isolation does not necessarily implicate them as the cause of the disease. Consequently, special methods to isolate opportunists become largely a waste of time and money. The geographical distribution of certain pathogens refers to their restriction to certain areas. The best example of this phenomenon is Vibrio cholerae, the cause of Asiatic cholera, which has its reservoir in Southern Asia (India, Pakistan, Burma, Nepal and China). Routine culture methods outside endemic areas do not include special media for its isolation. Lastly, since many microorganisms require special techniques for isolation, the number of pathogens that are actively searched for must be kept to a minimum. Otherwise, both the cost to the patient and the work required by the laboratory will reach intolerable levels. In light of the above facts, practical strategies for isolating enteric pathogens should involve using any special techniques necessary for the growth of pathogens known to occur in the area, and keeping a clinical and laboratory awareness for the possibility of an uncommon pathogen.

The classical bacterial enteric pathogens in the United States have been, until recently, members of the genera Salmonella and Shigella. These groups cause salmonellosis (which includes typhoid) and shigellosis (also known as bacillary dysentery), respectively. In recent

years, however, other bacteria have become implicated with regularity in enteric disease. These "newer" pathogens are members of the genera Yersinia and Campylobacter. The syndromes caused by these bacterial pathogens, although not usually as severe as those of typhoid (caused by Salmonella typhi) and bacillary dysentery (caused by Shigella dysenteriae), do rival those caused by the other species of Salmonella and Shigella, which are well-known and respected disease agents. In recent years more and more literature has expounded upon the syndromes, etiology, isolation and epidemiology of the "newer" enteric pathogens.

During the last two or three years, the three largest hospitals in Stockton (St. Joseph's, Dameron and San Joaquin General Hospitals) have modified their procedures to include the search for Campylobacter. On the other hand, the incidence of Yersinia in California is unknown, as most clinical laboratories do not specifically culture for it. There have been only a few reports of Yersinia in this state (personal communication of Dennis Ferrero, Director of the San Joaquin County Public Health Laboratory). This study has a threefold purpose: to investigate the incidences of Campylobacter and Yersinia, especially in relation to the incidence of Salmonella and Shigella, to study the biochemical and antibiogram characteristics of enteric isolates, particularly the "newer" pathogens, and to review the state of routine culture methods and assess their adequacy.

HISTORICAL REVIEW

Yersinia

The genus Yersinia, named in honor of the French bacteriologist A. J. E. Yersin, was proposed in 1944 by Van Loghem to accommodate the plague bacillus, previously known as Pasteurella pestis. In 1954 Thal proposed including Yersinia in the family Enterobacteriaceae. Mollaret and Thal (1974, p. 330) state "Numerical methods seem to justify the division of Pasteurella and the relation of Yersinia to the Enterobacteriaceae," conclusions based on the work of Smith and Thal (1965) and Talbot and Sneath (1960).

Another species in the genus Pasteurella that has been transferred to the genus Yersinia is P. pseudotuberculosis. The relationship between it and the plague bacillus, evident in cross-reactivity among their antigenic factors (Baker et. al., 1947) as well as sensitivity to the same phage types (Ben-Gurion and Hertman, 1958; Brubaker and Surgalla, 1962) and, more recently, the relatedness of their DNA (Bercovier et. al., 1980b), has been known for several decades. Yersinia pseudotuberculosis has been isolated from human tuberculosis-like lesions (thus its name) and infected mesenteric glands, as well as from the intestines and associated lymph nodes. Intestinal isolation, however, is not always associated with disease. This

species is serologically cross-reactive with several members of the Enterobacteriaceae. It was not formally placed in the genus Yersinia until 1965 by Smith and Thal.

Schleifstein and Coleman (1939) isolated a gram-negative bacillus from ulcers and cases of enterocolitis. The similarity of this organism, which they named Bacterium enterocoliticum, to Pasteurella pseudotuberculosis was subsequently established. Frederiksen, according to Bottone (1977, 1981), working in 1964 with cultures of Bacterium enterocoliticum from the 1939 study by Schleifstein and Coleman and with many isolates of related organisms from Europe, known as Pasteurella pseudotuberculosis type b, Pasteurella X and Germe X, performed biochemical and antibiotic tests. Frederiksen concluded that these isolates were similar enough to be placed in a single species. Because of certain similarities to Yersinia pseudotuberculosis, he placed the species in the genus Yersinia, and called it Yersinia enterocolitica.

"Because the organisms classified as Y. enterocolitica show different patterns of biochemical reactions, three schemes for dividing the species into biogroups were developed" (Shayegani et. al., 1981, p. 304): one by Nilèhn in 1969, the second by Wauters in 1970 and the third by Knapp and Thal in 1973. These were further modified by Brenner et. al. (1976, 1980a, 1980b), Bercovier et. al. (1980a, 1980c) and Ursing et. al. (1980), who proposed dividing the biogroups into species, based on DNA hybridiza-

tion and biochemical reactions. The name Y. enterocolitica was retained for those that showed typical biochemical reactions. The less typical strains were assigned to three new species, Y. frederiksenii, Y. intermedia and Y. kristensenii, and two unnamed groups currently referred to as X1 and X2. One other species is known, Y. ruckeri, a pathogen of rainbow trout and other fish. This species has not been implicated in human disease.

Yersinia infections include gastroenteritis, lymphadenitis, septicemia and several opportunistic sequelae. Epidemiological study of enterocolitica yersiniosis has implicated water, food and animals (swine, dogs, rabbits, chinchillas, cows, sheep, horses, deer, cats, beavers, raccoons, oysters and various birds) as reservoirs of the organism. The method of transmission and the roles of the other species (Y. intermedia, Y. frederiksenii, Y. kristensenii and groups X1 and X2) have not been fully determined.

Shigella

The genus Shigella was created in 1919 by Castellani and Chalmers to accommodate Bacillus dysenteriae Shiga, 1898 and related gram-negative, non-motile bacilli suspected of being the cause of bacillary dysentery.

According to Edwards and Ewing (1972, p. 108), agents of dysentery had been isolated prior to the work of Shiga.

Chantemesse and Widal (1888) reported the isolation of a bacterium from feces in five cases of acute dysen-

> tery. . . . Grigorieff (1891) isolated cultures from eleven cases of dysentery in Russia; he considered these to be identical with the bacterium described by Chantemesse and Widal (see Shiga, 1898).

Vaillard and Dopter, in 1903, found the cultures of Chantemesse and Widal to be the same as those described by Shiga in 1898. So although it is clear that the first isolation of dysentery-causing organisms precedes Shiga's report, Shiga's bacillus was selected as the type species.

A review of these earlier studies appears in Edwards and Ewing (1972). Today four species of Shigella are recognized. Their nomenclature and taxonomic scheme are based on reports by the Shigella Commission of 1950 with several amendments and excerpts by the International Enterobacteriaceae Subcommittee more recently. Three of the four species contain a number of serotypes: Sh. dysenteriae (10 serotypes), Sh. flexneri (6 serotypes and several subserotypes) and Sh. boydii (15 serotypes). Shigella sonnei occurs as a single serotype (Finegold et. al., 1978).

The identification of the species is based primarily on biochemical reactions followed by confirmation and identification of serotypes by serologic typing. Often the Shigella isolates are reported by serologic group rather than by species: Sh. dysenteriae as group A, Sh. flexneri, group B, Sh. boydii, group C and Sh. sonnei, group D. Shigella infections are now called shigellosis rather than bacillary dysentery.

In terms of severity of infections, Sh. dysenteriae produces the most severe form of shigellosis, Sh. sonnei the

mildest, and Sh. flexneri and Sh. boydii are intermediate. Sh. dysenteriae is rare in the United States, whereas Sh. sonnei infections are the most common.

Salmonella

The genus Salmonella was created in 1900 by Lignières in honor of D. E. Salmon, first chief of the U. S. Bureau of Animal Husbandry and codiscoverer (with Theobald Smith) of the gram-negative, motile bacillus known today as Salmonella cholerae-suis. Over 2,000 "species" are known as the cause of salmonellosis, including typhoid-paratyphoid. Actually these are antigenic types that have been assigned "species" status.

As the search for typhoid and paratyphoid organisms continued, serotyping became a basis for species identification. As a result, when a different antigenic type was found, it was given a new species name, usually based on the locality from which it was isolated (i.e. S. miami, S. singapore, S. panama, etc.). A method for a taxonomic organization of the species of Salmonella emerged in the form of the Kauffman-White schema. The schema divides the genus into "groups"--based on the kind(s) of somatic, or "O" antigens--and "types"--based on the kind(s) of flagellar, or "H" antigens. The many species names are retained as a matter of convenience and tradition. "Such names . . . are so well known and so universally used that it seems unwise to abandon them" (Kauffmann, 1954, p. 13).

Clearly, the Kauffman-White schema is a helpful

tool in delimiting the relationships among the salmonellae. However, the fact remains that not all of the serological entities declared in the schema are true species. Kauffmann and Edwards (1952) recognized three species of Salmonella: S. cholerae-suis (two serotypes) as type species, S. typhi (one serotype) and S. enteritidis (all other serotypes--currently over 2,000).

Kalz (1957, p. 369) states that "the suggestion by Kauffmann and Edwards to divide the genus into three species . . . appears too narrow and somewhat contradictory as it practically means that two type species are chosen." In defense of her own listing of ten species she further states (p. 369) "It seems justified to accord species rank to those organisms which are commonly encountered and/or cause rather well established syndromes." This is a reasonable approach and is followed in some fashion by many other authors such as Skerman et. al. (1980), who list five species of Salmonella: S. cholerae-suis, S. typhi, S. enteritidis, S. arizonae and S. typhimurium.

Campylobacter

The genus Campylobacter (campylo="curved," bacter="rod-shaped") was coined by Sebald and Véron in 1963 to place certain species of gram-negative, curved bacilli isolated from animal and human sources. These species were previously called Vibrio fetus and V. bubulus. Vibrio fetus, commonly isolated from cattle and sheep, was considered a pathogen, V. bubulus a commensal. The basis for the

new genus was the fact that these organisms differed in carbohydrate fermentation and DNA composition from the "true" vibrios, represented by Vibrio cholerae, the causal agent of Asiatic cholera (Sebald and Verón, 1963).

Organisms such as those currently found in the genus Campylobacter have been known since 1909 as the etiological agent of bovine contagious abortion. Other species of Vibrio from animals included V. sputorum, V. foetus-ovis and V. jejuni. The first human isolate of V. fetus was obtained by Vinzent, Dumas and Picard in 1947. This was followed by the isolation of the same species in the United States by Ward in 1948. These isolates of V. fetus from humans were from blood, cerebrospinal fluid and other body fluids.

King (1957) made the important discovery that there were two groups of V. fetus isolates, each with distinct serologic and biochemical characteristics. One of these was previously known as an opportunistic organism, isolated primarily from the elderly or those debilitated by alcoholism, malignant disease, diabetes mellitus or cardiovascular disease. The other, which she termed "related vibrios," grew best at 42C. She suggested that the "related vibrios" may be a cause of diarrheal illness and further speculated that they had not been isolated from stools because they were slow-growing and fastidious (King, 1957, 1962). Dekeyser et. al. (1972) confirmed her suspicion by isolating the "related vibrios" from diarrhetic stools using a selective medium.

According to "Approved Lists of Bacterial Names" (Skermen et. al., 1980), four species of Campylobacter are recognized: C. jejuni and C. coli as undivided species; C. fetus as two subspecies--subsp. fetus and subsp. venerealis; C. sputorum also as two subspecies--subsp. sputorum and subsp. bubulus. With the exception of the types species (C. fetus subsp. fetus, named by Sebald and Vèron in 1963) the above taxa were assigned to the genus by Vèron and Chatelain in 1973. Smibert (1974) lists one additional species, C. fecalis, and an additional subspecies, C. sputorum subsp. mucosalis. C. sputorum subsp. mucosalis is apparently a cause of ileitis in swine; C. fecalis and C. sputorum subsp. bubulus are bovine and ovine symbionts. C. sputorum subsp. sputorum is an oral symbiont of humans; C. fetus subsp. venerealis is a cause of sexually-transmitted bovine and ovine abortion. The remaining species (C. coli, C. jejuni and C. fetus subsp. fetus) live in the intestines of humans and animals and are the only pathogenic species of Campylobacter in humans.

Blaser and Reller (1981, p. 1445) summarize the evidence linking C. jejuni and diarrheal disease in humans as follows:

> In developed countries, C. jejuni has been isolated from the stools of 3 to 14 per cent of patients with diarrhea who seek medical attention, but only rarely has it been isolated from healthy persons. Outbreaks of gastrointestinal illness have occurred in which C. jejuni has been isolated from the stools of affected persons but not from those of persons who remained well, and no other pathogen has been found. Campylobacter has been isolated simultaneously from stool and blood cultures from some patients with enteritis. Patients

> with diarrheal illness from whom C. jejuni was the only pathogen isolated have had rising titers of serum antibodies specific for the infecting organism. Ill persons excreting C. jejuni who were treated with erythromycin--an agent to which most other recognized enteric pathogens do not respond--have rapid remissions of their symptoms, accompanied by clearance of the organism from their stools. Finally, in two separate experiments, volunteers who ingested cultures of C. jejuni in milk had gastrointestinal illness, and C. jejuni was isolated from their stools.

Blaser and Reller make reference to the species C. jejuni and C. coli together wherever the species C. jejuni is mentioned. This is because they "differ only slightly in phenotypic characteristics" (p. 1445).

MATERIALS AND METHODS

From January 1 to December 31, 1982, 973 specimens of stools and rectal swabs were cultured in the Dameron Hospital Microbiology Laboratory in Stockton, California. Each specimen was introduced directly into primary culture media and indirectly into secondary media from an enrichment broth. All media were purchased from Hana Media, Berkeley, California.

Isolation Media

Primary media. (See Appendix 1 for composition of all media) Each specimen was inoculated (Figure 1) directly, using standard isolation streaking techniques, into the following plated agar media:

1. BAP (Blood Agar Plate), an enriched medium used routinely with all clinical material.

2. EMB (Eosin-Methylene Blue), a routine differential/selective medium for the isolation of gram-negative bacilli.

3. HE (Hektoen Enteric), a differential/selective medium for the isolation of gram-negative enteric bacteria.

4. SS (*Salmonella-Shigella*), a selective medium for the isolation of the salmonellae and shigellae.

5. VPTK (Vancomycin-Polymixin B-Trimethoprim-Kanamycin), a selective medium for the isolation of *Campylobacter* species.

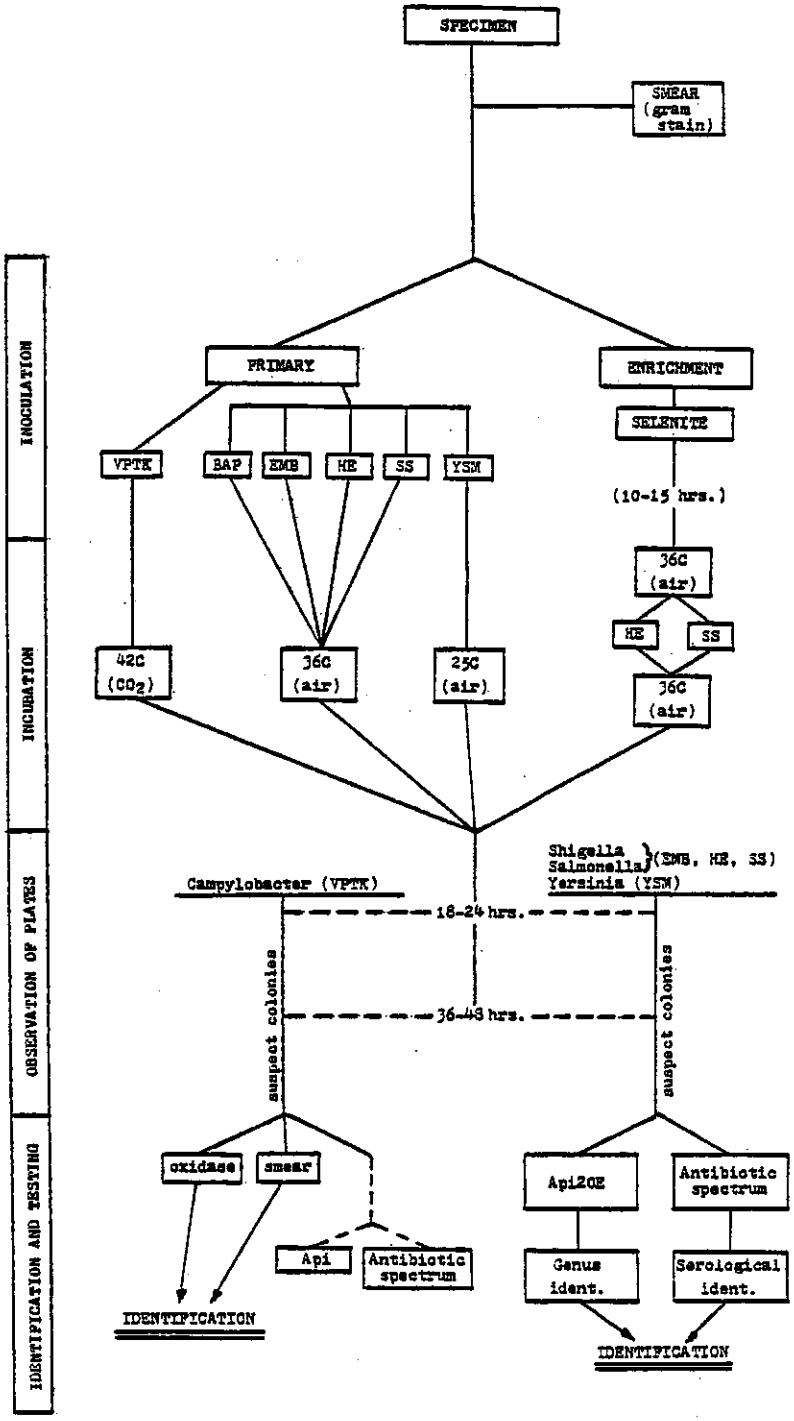

Figure 1

Procedures for Isolation and Identification of Enteric Pathogens

6. YSA (Yersinia Selective Agar + Antimicrobic Supplement CN), a selective medium for the isolation of Yersinia species.

Enrichment medium. In addition to inoculation of these primary isolation media, an enrichment medium, selenite broth, was used to enhance the growth of the salmonellae. After 10-15 hours in this medium, an inoculum was subcultured to SS and HE plates for subsequent incubation with the primary media.

Incubation Procedures

Blood Agar Plates, EMB, HE, SS and selenite broths were incubated aerobically at 35-37C and the YSA plates aerobically at 24-26C. The VPTK plates were incubated at 42C in a jar under increased CO_2 pressure and decreased O_2 tension, obtained by a Campy-Pak gas generator envelope (Baltimore Biological Laboratory, Cockeysville, Maryland).

Smears from Specimens

Along with these various cultivations, smears were prepared, stained with the gram stain technique and examined under oil-immersion microscopy to determine the presence or absence of white blood cells and the distribution of intestinal microbial flora.

Presumptive Identification of Colonies

Blood Agar Plates are used as a routine culture medium for the isolation of gram-positive and gram-negative

bacteria. Colonies of gram-negative enteric bacteria have no distinctive appearance on BAP. After 18-24 hours and again after 36-48 hours of incubation, all plates (EMB, HE, SS, VPTK and YSA) are examined for the presence of colonies suspected of being enteric pathogens.

Salmonella. Colonies of Salmonella appear bluish-green on HE, often with a black center, and are colorless on EMB and SS. Salmonella will not grow in VPTK or YSA.

Shigella. Colonies of Shigella also appear bluish-green on HE, but always without a black center. Like Salmonella, Shigella is colorless on EMB and SS, and will not grow in VPTK or YSA.

Campylobacter. Colonies of Campylobacter appear grayish and mucoid on VPTK. Campylobacter will not grow in the other media unless they are incubated microaerophilically (decreased O_2 and increased CO_2).

Yersinia. Colonies of Yersinia appear pink on YSA, and colorless or pink on HE, EMB and SS. Yersinia will not grow on VPTK.

Definitive Identification of Isolates

Colonies suspected of being pathogens were further tested to confirm their identities.

Biochemical testing. With the exception of Campylobacter species, all suspected pathogens were identified

using the biochemical array of the Api20E (Analytab Products, Plainview, New York), a kit consisting of 20 tests (see Table 1). Appendix 2 gives the biochemical principles for the tests of the Api20E. This system was inoculated according to the manufacturer's instructions, using a saline suspension of growth derived from a single colony taken from one of the isolation media, and was subsequently incubated at 35-37C for 24 hours.

Campylobacter. Definitive identification of Campylobacter jejuni was based on growth on VPTK plates at 42C, a positive reaction for oxidase (Patho-tec CO strips, General Diagnostics, Morris Plains, New Jersey) and on the demonstration of curved bacilli under oil immersion microscopy. Some of the isolates with the above characteristics were inoculated into the Api20E (seven isolates) and the Api20A (ten isolates).

The Api20A differs from the Api20E in that the former kit is designed for testing anaerobic bacteria (Table 2). The difference in usage of the Api20A lies in suspending the single colony in thioglycollate broth and incubating under microaerophilic conditions at 42C. Appendix 3 shows the biochemical principles for the tests of the Api20A. The Api20E kits used for testing Campylobacter were also incubated microaerophilically at 42C.

Antimicrobial Testing of Isolates

Antimicrobial testing was performed using the

Table 1

Api20E Biochemical Tests

abbreviation	test
ONPG	O-nitrophenyl-β-d-galactosidase
ADH	arginine dihydrolase
LDC	lysine decarboxylase
ODC	ornithine decarboxylase
CIT	citrate utilization
H_2S	hydrogen sulfide production
URE	urea hydrolysis
TDA	tryptophane deaminase
IND	indole production
VP	Voges-Proskauer test
GEL	gelatin hydrolysis
GLU	glucose fermentation
MAN	mannitol fermentation
INO	inositol fermentation
SOR	sorbitol fermentation
RHA	rhamnose fermentation
SAC	saccharose (sucrose) fermentation
MEL	melibiose fermentation
AMY	amygdalin fermentation
ARA	arabinose fermentation

Table 2

Api20A Biochemical Tests

abbreviation	test
IND	indole production
URE	urea hydrolysis
GLU	glucose fermentation
MAN	mannitol fermentation
LAC	lactose fermentation
SAC	saccharose (sucrose) fermentation
MAL	maltose fermentation
SAL	salicin fermentation
XYL	d (+) xylose fermentation
ARA	l (+) arabinose fermentation
GEL	gelatin hydrolysis
ESC	esculin hydrolysis
GLY	glycerol fermentation
CEL	cellobiose fermentation
MNE	mannose fermentation
MLZ	melizitose fermentation
RAF	raffinose fermentation
SOR	sorbitol fermentation
RHA	rhamnose fermentation
TRE	trehalose fermentation

Kirby-Bauer standardized disc diffusion method. Portions of 3-5 colonies from one of the isolation media were transferred to a 10ml Mueller-Hinton broth and incubated at 35-37C until a turbidity equivalent to a No. 5 McFarland nephelometer barium sulfate standard (0.5ml 1% aqueous barium chloride, 9.5ml 1% sulfuric acid) was reached, as measured by a nephelometer (Analytab Products, Plainview, New York). For Campylobacter, portions of 3-5 colonies were transferred to a 10ml thioglycollate broth until the desired turbidity was achieved. No incubation was done in the preparation of Campylobacter broths. Following inoculation of the entire surface of a Mueller-Hinton Agar Plate (Mueller-Hinton Chocolate Agar for Campylobacter) from one of the above broths, paper discs impregnated with antimicrobial agents (Difco Laboratories, Detroit, Michigan) were placed on the agar surface. See Tables 9, 10 and 11 (pages 35, 36 and 37, respectively) for the antimicrobial agents used. The inoculated plates were then incubated aerobically at 35-37C (42C under microaerophilic conditions for Campylobacter) for 24 hours. Because of the cost involved, only 24 Campylobacter strains were tested.

Serological Testing of Isolates

Isolates identified as either Salmonella or Shigella species were further identified to serogroups by slide agglutination of somatic antigens. For this procedure antisera (Difco Laboratories, Detroit, Michigan) of various antigenic groups were mixed with cells taken from the

Mueller-Hinton plates. Confirmation and further serological testing were provided by the San Joaquin Health Center Microbiology Laboratory on all isolates of Salmonella and Shigella except Shigella sonnei.

Computer Analysis of Data

A computer program was written to analyze the data and information with regards to patients' ages and sexes, monthly distribution of the positive cultures, biochemical results and antibiotic susceptibilities. The program was written in Pascal programming language, compiled and run with the Burroughs 6700 mainframe computer at the University of the Pacific Computer Center.

Statistical Analysis of Data

The chi-squared test was used to determine the level of significance of certain distributions of infected patients with respect to sex, age and month of culture.

RESULTS

Positive Cultures

Of the 973 stools and rectal swabs cultured, 132 (13.8%), involving 123 patients, were positive, yielding a total of 134 pathogens; two positive cultures from different patients showed mixed infections of *Campylobacter jejuni* and *Shigella sonnei*. Table 3 shows the distribution of the pathogens by species: 31 isolates of *Shigella* (23% of all pathogens), 21 isolates of *Salmonella* (16%) and 82 isolates of *C. jejuni* (61%). No *Yersinia* species were isolated.

Shigella. *Shigella sonnei* was the predominant organism among the shigellas (23 isolates or 43%). Two other species in the genus were encountered: *Sh. flexneri* (6 or 20%) and *Sh. boydii* (2 or 6%). *Shigella dysenteriae*, a species endemic and common in the Far East but rare in the United States, was not found.

Salmonella. *Salmonella typhimurium* was the most common of the salmonellas (9 isolates or 43%). Six other species of *Salmonella* were identified: three *S. enteritidis*, one each of *S. give*, *S. heidelberg* and *S. mbundaka* and two each of *S. newport* and *S. typhi*. Two isolates were not identified to species.

Table 3

Enteric Pathogen Isolates

pathogen	number of isolates[a]	rate from all cultures[b]	pct. of all pathogens[c]
Shigella			
sonnei	23	2.4	17
flexneri	6	.6	4.5
boydii	2	.2	1.5
total *Shigella*	31	3.2	23
Salmonella			
typhimurium	9	.9	6.7
enteritidis	3	.3	2.2
give	1	.1	.7
heidelberg	1	.1	.7
mbundaka	1	.1	.7
newport	2	.2	1.5
sp.	2	.2	1.5
typhi	2	.2	1.5
total *Salmonella*	21	2.2	16
Campylobacter			
jejuni	82	8.4	61
all pathogens	134	13.8	100

[a]Of 123 infected patients, seven were cultured more than once.

[b]The number of isolates as a percentage of the total number of cultures.

[c]The number of isolates as a percentage of the total number of pathogens.

Campylobacter. Only Campylobacter jejuni was encountered. The 82 isolates represent 61% of all the bacterial enteric infections in this study.

Distribution of Patients by Sex

The distribution, with respect to sex, of patients with positive cultures is shown in Table 4. The discrepancy in numbers (125 vs. 123 total patients) is due to mixed infections involving two patients.

A chi-squared analysis of the differences between the number of males and females infected by each species was done. When the observed number of infections was compared to the expected number of infections (based on the number of cultures performed on each sex), no statistical signigicance was found for Shigella ($p < .80$), Salmonella ($p < .80$) or Campylobacter ($p < .20$).

Distribution of Patients by Age

Table 5 (page 27) shows the distribution of infected patients by age. In the genus Shigella, 78% of Sh. sonnei hosts were age ten years of less ($p < .50$). Only five infections (22%) were in patients older than 16 years (17, 21, 27, 36 and 39 years). The three patients with Sh. flexneri were 2, 4 and 60 years old; those with Sh. boydii were 6 and 12 years of age.

The Salmonella infections followed much the same pattern, involving mostly young people and infants. Of all patients with Salmonella, 88% were 12 years of age or

Table 4

Distribution, According to Sex, of Patients with Enteric Pathogens

pathogen	no. patients[a]	male	female	not known[b]
Shigella				
sonnei	23[c]	10[c]	13	0
flexneri	3	2	1	0
boydii	2	0	2	0
total *Shigella*	28	12	16	0
Salmonella				
typhimurium	7	4	3	0
enteritidis	3	2	0	1
give	1	1	0	0
heidelberg	1	0	1	0
mbundaka	1	0	1	0
newport	1	0	1	0
sp.	2	0	1	1
typhi	1	1	0	0
total *Salmonella*	17	8	7	2
Campylobacter				
jejuni	80[c]	44[c]	31	5
all pathogens	125	64	54	7
no. cultures	-	436	464	73

[a]Number of patients showing one or more positive cultures.

[b]Patient sex was not indicated and/or could not be determined from the name.

[c]Two patients had double infections involving *Sh. sonnei* and *C. jejuni*. These patients were counted once for each of the species isolated.

younger ($p < .10$). The two oldest patients that had Salmonella infections were a 19-year- and a 41-year-old; both were infected with S. typhimurium. The other five patients with S. typhimurium were less than six years old.

In contrast to the age bias of the Salmonella and Shigella infections, Campylobacter infections were found to be well represented in all age groups, from two months to 87 years. In the 19-to-55-year age group 26 (33%) of the C. jejuni infections were found; 25 (31%) were in two-year-olds and under; in between these two groups were 22 (28%) of the C. jejuni infections. Four patients with C. jejuni were of unknown age.

Monthly Distribution of Infections

The monthly distribution of the enteric infections is given in Table 6. All infections by Sh. sonnei occurred in a span of roughly five months--July 20 to December 25 ($p < .001$). Furthermore, 93% of all Shigella infections (all Sh. sonnei and Sh. flexneri) appeared from June to December ($p < .001$); the two Sh. boydii infections occurred in February and April.

The distribution of the salmonellae is also seasonal, but seems to occur mostly in the first part of the year. All S. typhimurium were isolated during the five-month period from February 4 to July 5 ($p < .01$). Furthermore, except for the two Salmonella sp. isolations in September and November, all other species of Salmonella (88%) were recovered between February and July ($p < .001$).

Table 5

Age Distribution of Patients with Enteric Infections

pathogen	no. patients[a]	under 6 months	6-24 months	3-10 years	11-18 years	19-55 years	over 56 years	age unknown
Shigella								
sonnei	23	0	7[b]	11[b]	1	4	0	0
flexneri	3	0	1	1	0	0	1	0
boydii	2	0	0	1	1	0	0	0
total *Shigella*	28	0	8	13	2	4	1	0
Salmonella								
typhimurium	7	2	2	1	0	2	0	0
enteritidis	3	1	1	0	1	0	0	0
give	1	0	1	0	0	0	0	0
heidelberg	1	1	0	0	0	0	0	0
mbundaka	1	0	0	1	0	0	0	0
newport	1	1	0	0	0	0	0	0
sp.	2	1	1	0	0	0	0	0
typhi	1	0	1	0	0	0	0	0
total *Salmonella*	17	6	6	2	1	2	0	0
Campylobacter								
jejuni	80	6	19[b]	14[b]	8	26	3	4
all pathogens	125	12	33	29	11	32	4	4
no. cultures	-	218	300	95	27	201	81	51

[a]See Table 4, footnote a.

[b]See Table 4, footnote c.

Table 6

Monthly Distribution of Enteric Infections

pathogen	patients[a]	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Shigella													
sonnei	23	-	-	-	-	-	-	2[b]	8[b]	2	3	4	2
flexneri	3	-	-	-	-	-	1	1	-	1	-	-	-
boydii	2	-	1	-	1	-	-	-	-	-	-	-	-
total Shigella	28	-	1	-	1	-	1	3	8	3	3	4	4
Salmonella													
typhimurium	7	-	1	1	3	-	1	1	-	-	-	-	-
enteritidis	3	-	-	1	-	-	1	1	-	-	-	-	-
give	1	-	-	-	-	1	-	-	-	-	-	-	-
heidelberg	1	-	-	-	-	-	-	1	-	-	-	-	-
mbundaka	1	-	-	-	1	-	-	-	-	-	-	-	-
newport	1	-	-	-	1	-	-	-	-	-	-	-	-
sp.	2	-	-	-	-	-	-	-	-	1	-	1	-
typhi	1	-	-	-	-	-	-	1	-	-	-	-	-
total Salmonella	17	-	1	2	5	1	2	4	-	1	-	1	-
Campylobacter													
jejuni	80	8	2	2	5	7	10	12[b]	8[b]	5	6	6	9
all pathogens	125	8	4	4	11	8	13	19	16	9	9	11	13
no. cultures	-	54	80	53	44	72	106	98	79	94	113	89	91

[a]See Table 4, footnote a.

[b]See Table 4, footnote c.

Campylobacter jejuni contrasts with the previous genera in that its infections were present in every month of the year. Its rate of occurrence with respect to the number of cultures performed in each month fluctuated over the course of the year, but in terms of the number of infections, there was a four-month period (June 5 to September 4) in which 40% of C. jejuni infections arose.

Biochemicals

Occasionally more than one Api kit was used for testing isolates from a given set of media, especially when suspicious colonies appeared on more than one of the plates.

Shigella. All strains of Shigella sonnei had a common baseline of five positive biochemical tests (Table 7): beta-galactosidase (ONPG), ornithine dihydrolase, glucose, mannitol and arabinose. The most frequent biochemical pattern of this species (profile number 1-104-112) also showed rhamnose fermentation, and appeared at a rate of 48%. In contrast, the next most common pattern (profile number 1-104-102), present 45% of the time, was rhamnose-negative. Two other patterns (profile numbers 1-104-152 and 1-104-512) were each expressed by a single isolate and each displayed a single difference from the baseline: the former, melibiose fermentation, the latter, sorbitol fermentation.

For Sh. flexneri two patterns (profile numbers 0-004-100 and 0-004-102), involving two isolates each,

Table 7

Biochemical Patterns of the Shigellae

species	profile no.[a]	no. occurrences of pattern[b]	biochemical test[c]																			
			ONPG	ADH	LDC	ODC	CIT	H_2S	URE	TDA	IND	VP	GEL	GLU	MAN	INO	SOR	RHA	SAC	MEL	AMY	ARA
Sh. sonnei	1-104-112	15	+	-	-	+	-	-	-	-	-	-	-	+	+	-	-	+	-	-	-	+
	1-104-102	14	+	-	-	+	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	+
	1-104-152	1	+	-	-	+	-	-	-	-	-	-	-	+	+	-	-	+	-	+	-	+
	1-104-152	1	+	-	-	+	-	-	-	-	-	-	-	+	+	-	+	+	-	-	-	+
	percentage of positive reactions:		100	0	0	100	0	0	0	0	0	0	0	100	100	0	3	55	0	3	0	100
Sh. flexneri	0-004-100	2	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-
	0-004-102	2	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	+
	percentage of positive reactions:		0	0	0	0	0	0	0	0	0	0	0	100	100	0	0	0	0	0	0	50
Sh. boydii	0-004-102	2	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	+
	percentage of positive reactions:		0	0	0	0	0	0	0	0	0	0	0	100	100	0	0	0	0	0	0	100

[a] See Appendix 4 for interpretation of profile numbers.

[b] Some isolates were tested more than once.

[c] See Table 1 for abbreviations of biochemical tests.

+ Positive reaction.

- Negative reaction.

were observed. Both demonstrated glucose and mannitol fermentation, and the latter profile was also positive for arabinose.

The two strains of Sh. boydii were positive for glucose, mannitol and arabinose (profile number 0-004-102). Although this species had the same profile number as did two isolates of Sh. flexneri, the identities of all Shigella were confirmed by serological testing, and so the biochemical results represent the variability that is inherent in any biochemical testing.

Salmonella. Definitive identification of Salmonella isolates is based on serological testing and not on biochemical reactions, with the exception of S. typhi, which can be distinguished from the other species by serological as well as biochemical characteristics.

Six biochemical patterns were noted for Salmonella typhimurium (Table 8). The baseline of tests that were positive for all strains included eight tests: ornithine decarboxylase, hydrogen sulfide and the fermentation of six carbohydrates--glucose, mannitol, sorbitol, rhamnose, melibiose and arabinose. The pattern which appeared most often (55%) was also positive for lysine decarboxylase and inositol (profile number 4-504-752). The other five patterns, each exhibited by a single strain, differed almost exclusively in tests other than carbohydrate fermentation. Of these, one strain was positive for both beta-galactosidase and indole (profile number 5-744-752), characteristics that

Table 8

Biochemical Patterns of the Salmonellae

species	profile no.[a]	no. occurrences of pattern[b]	biochemical test[c]																				
			ONPG	ADR	LDC	ODC	CIT	H_2S	URE	TDA	IND	VP	GEL	GLU	MAN	INO	SOR	RHA	SAC	MEL	AMY	ARA
S. typhimurium	4-504-752	6	-	-	+	+	-	+	-	-	-	-	-	+	+	+	+	+	-	+	-	+
	2-594-752	1	-	+	-	+	-	+	-	-	-	-	-	+	+	+	+	+	-	+	-	+
	0-504-752	1	-	-	-	+	-	+	-	-	-	-	-	+	+	+	+	+	-	+	-	+
	4-704-552	1	-	-	+	+	+	+	-	-	-	-	-	+	+	-	+	+	-	+	-	+
	5-744-752	1	+	-	+	+	+	+	-	-	+	-	-	+	+	+	+	+	-	+	-	+
	6-504-752	1	-	+	+	+	-	+	-	-	-	-	-	+	+	+	+	+	-	+	-	+
	percentage of positive reactions:		9	18	81	100	18	100	0	0	9	0	0	100	100	91	100	100	0	100	0	100
S. enteritidis	4-504-552	1	-	-	+	+	-	+	-	-	-	-	-	+	+	-	+	+	-	+	-	+
	4-504-572	1	-	-	+	+	-	+	-	-	-	-	-	+	+	-	+	+	+	+	-	+
	4-704-552	1	-	-	+	+	+	+	-	-	-	-	-	+	+	-	+	+	-	+	-	+
	percentage of positive reactions:		0	0	100	100	33	100	0	0	0	0	0	100	100	0	100	100	33	100	0	100
S. give[d]	percentage of positive reactions:		0	0	100	100	100	100	0	0	0	0	0	100	100	100	100	100	0	100	0	100
S. heidelberg[d]	percentage of positive reactions:		0	0	100	100	0	100	0	0	0	0	0	100	100	0	100	100	0	100	0	100
S. abundaka[d]	percentage of positive reactions:		0	0	100	100	100	100	0	0	0	0	0	100	100	100	100	100	0	100	0	100
S. newport[d]	percentage of positive reactions:		0	0	100	100	0	100	0	0	0	0	0	100	100	0	100	100	0	100	0	100
S. sp.	0-504-752	1	-	-	-	+	-	+	-	-	-	-	-	+	+	+	+	+	-	+	-	+
	0-704-552	1	-	-	+	+	+	+	-	-	-	-	-	+	+	-	+	+	-	+	-	+
	percentage of positive reactions:		0	0	50	100	50	100	0	0	0	0	0	100	100	50	100	100	0	100	0	100
S. typhi	percentage of positive reactions:		0	0	100	0	0	0	0	0	0	0	0	100	100	0	100	0	0	0	0	0

[a] See Appendix 4 for interpretation of profile numbers.

[b] Some isolates were tested more than once.

[c] See Table 1 for abbreviations of biochemical tests.

[d] Tested only once.

+ Positive reaction. - Negative reaction.

are extremely rare for species of Salmonella.

Each of the three isolates of S. enteritidis had a different pattern. The differences between these strains were in saccharose (sucrose) fermentation (one strain) and citrate utilization (one strain). The positive tests common to all S. enteritidis isolates were demonstrated by the third strain (profile number 4-504-552).

The two isolates identified as Salmonella sp. were quite different from each other, as is to be expected since these might be any of the 2,000-plus species in the genus. All other species of Salmonella were tested only once.

Campylobacter. Campylobacter jejuni was tested with the Api20A (ten isolates) as well as the Api20E (seven isolates). This species was biochemically inert after 24 hours in these systems. A slightly positive result was observed after 48 hours for the arginine dihydrolase test (Api20E) and after 48-72 hours for the urease and gelatinase tests (Api20A). All C. jejuni were oxidase-positive (Patho-tec strips).

Antimicrobials

Unlike the biochemical tests, which are all provided in every Api kit, the different types of antibiotic discs are packaged separately. Consequently, in many instances certain antibiotics were not used in susceptibility testing, due to occasional human error or the depletion of supplies.

Shigella. The information given in Table 9 shows that Shigella species were susceptible to most antibiotics and that resistance or intermediate susceptibility usually occurred to the same drugs in all species. Thus, all species included some strains resistant to tetracycline and gantrisin, and at least two of the three species evidenced some strains that were resistant and/or intermediate for ampicillin, carbenicillin, kanamycin and neomycin.

An interesting phenomenon concerning Sh. sonnei is that all strains which fermented rhamnose in the biochemical testing (profile numbers 1-104-112, 1-104-152 and 1-104-512) were resistant to gantrisin, and vice versa.

Salmonella. A comparison of Tables 9 and 10 shows that the susceptibilities of the salmonellae were similar to those of the shigellae in that both exhibted resistant and/or intermediate strains to the same drugs. In contrast, Salmonella species showed fewer strains that were resistant to gantrisin and/or tetracycline. Salmonella typhimurium was resistant to more drugs than all other species in the genus combined.

With respect to both Salmonella and Shigella, resistance to ampicillin and carbenicillin always occurred together.

Campylobacter. The Kirby-Bauer disc-diffusion method is not standardized for Campylobacter jejuni. An approximation for interpreting the inhibition zones of this

Table 9

Antimicrobial Susceptibility of the Shigellae[a/b]

	Sh. sonnei	Sh. flexneri	Sh. boydii
strains tested	23	3	2
ampicillin	96	100	50
carbenicillin	96	100	50
piperacillin	100	100	100
cephalothin	100	100	100
cefamandole	100	100	100
cefoxitin	100	100	100
cefotaxime	100	100	100
cefoperazone	100	100	100
moxalactam	100	100	100
tetracycline	27	50	0
gentamicin	100	100	100
kanamycin	87/9	67/33	100
amikacin	100	100	100
tobramycin	100	100	100
neomycin	94	33/67	100
polymyxin B	100	100	100
chloramphenicol	100	100	100
gantrisin	41	33	50
bactrim-septra	100	100	100

[a]Figures shown represent percentage of susceptible strains.

[b]Percentage of intermediate strains, if any, is given after the slash mark.

Table 10

Antimicrobial Susceptibility of the Salmonellae[a/b]

	S. typhimurium	S. enteritidis	S. give	S. heidelberg	S. mbundaka	S. newport	S. sp.	S. typhi
strains tested	8	3	2	1	1	1	1	1
ampicillin	75	100	100	100	100	100	100	100
carbenicillin	75	100	100	100	100	100	100	100
piperacillin	100	100	100	-	-	-	100	100
cephalothin	100	100	100	100	100	100	100	100
cefamandole	100	100	100	100	100	100	100	100
cefoxitin	100	100	100	100	100	100	100	100
cefotaxime	100	100	100	100	100	100	100	100
cefoperazone	100	100	100	100	100	100	100	100
moxalactam	100	100	100	100	100	100	100	100
tetracycline	50/25	100	50	100	100	100	0/100	100
gentamicin	100	100	100	100	100	100	100	100
kanamycin	75	100	100	100	100	100	100	100
amikacin	100	100	100	100	100	100	100	100
tobramycin	100	100	100	100	100	100	100	100
neomycin	75	100	100	100	-	100	100	-
polymyxin B	100	100	100	100	100	100	100	100
chloramphenicol	100	100	100	100	100	100	100	100
gantrisin	75	100	100	100	100	100	0/100	100
bactrim-septra	100	100	100	100	100	100	100	100

[a/b]See Table 9, footnotes a and b.

species may be obtained by using the values that appear in the literature for determining the susceptibilities of the Enterobacteriaceae, as was done for Table 11. By these standards it is apparent that six drugs were ineffective against C. jejuni, with little or no susceptibility. On the other hand, five drugs (gentamicin, amikacin, chloramphenicol, naladixic acid and erythromycin) worked quite well, showing 100% susceptibility; three other drugs (kanamycin, tobramycin and neomycin) exhibited over 80 percent susceptibility with the remaining strains intermediate.

Further insight as to the in vitro response of C. jejuni to antimicrobials may be gained by studying the inhibition zones themselves, their ranges and their averages. Such information is provided in Table 12. Thus it can readily be seen that this species must be resistant to cephalothin, cefamandole, cefoxitin and cefoperazone since no zone of inhibition was produced. Other antimicrobials that failed with regularity against C. jejuni were moxalactam and bactrim-septra. On the other hand, seven drugs (carbenicillin, gentamicin, amikacin, tobramycin, chloramphenicol, naladixic acid and erythromycin) were very effective, consistently producing large zones (18mm or larger). All other antimicrobials produced mixed results.

Table 11

Antimicrobial Susceptibility of *Campylobacter jejuni*[a/b]

	C. jejuni
strains tested	24
ampicillin	75/17
carbenicillin	65/35
piperacillin	56/13
cephalothin	0
cefamandole	0
cefoxitin	0
cefotaxime	67/29
cefoperazone	0
moxalactam	4/38
tetracycline	83
gentamicin	100
kanamycin	94/6
amikacin	100
tobramycin	83/17
neomycin	95/5
chloramphenicol	100
bactrim-septra	4/9
naladixic acid	100
erythromycin	100

[a/b] See Table 9, footnotes a and b.

Table 12

Inhibition Zones of 24 Strains of *Campylobacter jejuni* to 18 Antimicrobial Agents[a]

strain	Penicillins			Cephalosporins						Tetracycline	Amino-glycosides					Chloramphenicol	Bactrim-septra	Naladixic acid	Erythromycin
	Ampicillin	Carbenicillin	Piperacillin	Cephalothin	Cefamandole	Cefoxitin	Cefotaxime	Cefoperazone	Moxalactam		Gentamicin	Kanamycin	Amikacin	Tobramycin	Neomycin				
(1)	18	22	-	0	0	0	28	0	18	34	-	-	-	-	24	36	0	-	34
(2)	20	22	-	0	0	0	24	0	15	30	-	-	-	-	20	28	0	-	20
(3)	20	26	-	0	0	0	26	0	28	26	-	-	-	-	18	30	0	-	20
(4)	10	-	10	0	0	0	13	0	0	32	32	20	26	15	24	30	30	-	30
(5)	20	25	18	0	0	0	24	0	18	27	27	22	26	22	25	35	0	27	34
(6)	20	22	18	0	0	0	20	0	10	22	20	18	18	17	16	20	0	-	-
(7)	21	32	21	0	0	0	25	0	12	9	30	25	28	23	22	35	0	26	30
(8)	22	27	22	0	0	0	22	0	21	35	29	22	25	21	25	35	0	27	31
(9)	15	24	15	0	0	0	24	0	10	8	28	24	31	24	28	35	9	24	34
(10)	21	27	23	0	0	0	19	0	11	-	-	-	-	-	-	-	-	27	33
(11)	22	28	22	0	0	0	24	-	0	34	-	-	-	-	-	30	0	26	-
(12)	15	25	19	0	0	0	26	0	10	39	33	26	31	24	30	36	14	29	39
(13)	24	32	22	0	0	0	31	0	14	39	32	22	28	20	32	33	9	28	37
(14)	13	24	12	0	0	0	28	0	17	9	29	20	28	23	29	35	0	24	32
(15)	20	26	20	0	0	0	26	0	16	34	26	20	24	18	24	36	0	-	30
(16)	22	32	-	0	0	0	29	0	17	36	31	26	33	24	26	36	0	28	33
(17)	12	29	-	0	0	0	19	0	0	34	26	24	31	24	21	32	0	27	34
(18)	11	20	-	0	0	0	20	0	8	32	27	24	29	20	22	31	0	24	31
(19)	12	20	11	0	0	0	17	0	0	30	24	20	26	18	20	29	0	21	26
(20)	13	23	-	0	0	0	21	0	10	32	28	24	29	20	23	31	0	24	31
(21)	18	28	-	0	0	0	23	0	16	30	23	19	25	14	22	30	0	26	31
(22)	24	30	12	0	0	-	22	0	0	24	24	20	25	14	24	26	15	-	26
(23)	18	22	0	0	0	0	18	0	0	34	-	-	-	-	24	36	0	-	34
(24)	20	22	15	0	0	0	25	0	18	0	24	16	22	14	26	30	0	-	30
range	10-24	19-32	0-23	0	0	0	13-31	0	0-21	0-39	20-33	16-26	18-33	24-34	16-26	20-36	0-30	21-29	20-39
average	18	25	17	0	0	0	23	0	13	28	17	22	27	20	24	32	8	26	31

[a]Zones are given in mm.

- Indicates that the drug was not used.

DISCUSSION

This is the first study in San Joaquin County in which an attempt to isolate Yersinia enterocolitica was made, and a comparison of the incidence of Campylobacter species to that of Salmonella and Shigella was documented. Few laboratories in the State of California are currently culturing for Yersinia and Campylobacter. Government regulations concerning these "newer" pathogens have not been updated to include the collection of data on a routine basis. Public Health regulations require all clinical laboratories involved in enteric bacteriology to report the isolations of Salmonella and Shigella, and to submit a slant culture of each isolate for confirmation and definitive identification (The only exception to this is Shigella sonnei, the most common but least pathogenic cause of shigellosis in the United States). Federal and State Health regulations do not require a report of incidences or a submission of cultures of any Yersinia or Campylobacter (except Yersinia pestis, the causative agent of plague).

Yersinia

Several reports (Greenwood et. al., 1975; Bissett, 1976; Bottone, 1977, 1981; Chester et. al., 1981; Shayegani et. al., 1981; Sonnenwirth, 1981; Weissfeld, 1981; Lofgren et. al., 1982) concerning the isolation of Yersinia enterocolitica in various parts of the United States have

been made recently. Several of these have suggested a geographical distribution associated with cold environments. However, the report by Bissett (1976) on 24 isolates from California, and that by Chester et. al. (1981) on three recoveries made in Florida, indicate the existence of infections outside of such climates.

The methods used in the past to attempt isolation of Y. enterocolitica from stools are now suspected to be inadequate and part of the reason for its low isolation rate. Improved methods that are currently in use include cold enrichment (Greenwood et. al., 1975), KOH treatment (Weissfeld and Sonnenwirth, 1981) and the use of better selective media such as Yersinia Selective Agar (YSA). The success of cold enrichment is well documented, but suffers from the problem of a 10-21 day enrichment period at 4C, which is practical only for epidemiological studies, and not for clinical situations in which infections generally last less than 10 days. The result of this study concerning the use of YSA as a direct plating medium indicates only that Y. enterocolitica was not isolated. A study in which the methods of KOH treatment and the use of selective media are compared to the proven method of cold enrichment may give more definitive results reflecting the effectiveness of the various methods.

Shigella

The incidence of Shigella sonnei was far greater than that of the rest of the shigellae put together. This

is as expected. The interesting features of Sh. sonnei infections include their predominance in young and infantile patients (even though more cultures were performed on this group than any other), and their exclusive occurrence in the latter half of the year. Shigellosis prevalence in the young is well documented in Bac Data computerized reports (personal communication of F. M. Nahhas, Director of the Dameron Hospital Microbiology Laboratory), but no particular seasonal distribution of this infection was encountered in the literature.

Biochemically, the Shigella species reacted with predictability. The percentages of positive reactions (Table 7, p. 30) were compared to those of the Api data base. The only observable difference between these sets of figures is the fermentation of melibiose by one strain of Sh. sonnei. The Api data, based on 740 strains, show a zero percent fermentation of this carbohydrate. This apparent anomaly may be a rare reaction, or could be due to the contamination of that particular test.

Antimicrobial testing of Shigella revealed nothing new. Ampicillin, the drug of choice in treating enteric infections, was largely effective, in vitro, with a few exceptions--one Sh. sonnei of 23 and one of two isolates of Sh. boydii. Seventy-three percent of Sh. sonnei and 50% of Sh. flexneri were resistant to tetracycline, and more than 50 percent of all the shigellas were resistant to gantrisin (sulfa). A review by the World Health Organization Scien-

tific Working Group (1980) indicates similar patterns of resistance in Shigella worldwide, and suggests that multiple drug resistance is plasmid-mediated and probably "related to the unrestricted sale and use of antibiotics in man" (p. 533).

A comparison of antibiotic and biochemical patterns on a strain-by-strain basis disclosed the peculiar association of a drug resistance and a carbohydrate fermentation. Thirteen of the 23 isolates of Sh. sonnei were rhamnose fermenters (some positive cultures were tested more than once to yield a total of 17 rhamnose-positive strains). The antimicrobial test of Sh. sonnei revealed 13 strains that were resistant to gantrisin. These 13 resistant strains corresponded exactly with the 13 isolates that fermented rhamnose. Neither the mechanism nor the significance of these events is known.

Salmonella

A description of the incidence of Salmonella may take several forms, depending on the assumed attitude toward the classification of the genus. From an epidemiological standpoint it is pragmatic to make use of the well-known "species" names, numerous though they may be, as well as the serological group designations of the salmonellae. This study, however, was concerned mainly with the use of the species names alone, and consequently several infections were attributed to such obscure names as Salmonella heidelberg and Salmonella mbundaka, which have little meaning in

a study of this kind. Perhaps a more useful approach would be to group the organisms together and assess their results generically.

The incidence of the salmonellae was less frequent but similar in some ways to that of the shigellae. Considering age distribution, salmonellosis, like shigellosis, was found to be primarily a disease of infants and young children. The occurrence of salmonellosis during the year was, like shigellosis, apparently a seasonal one, although it occurred mostly in the first half of the year.

The two isolates (one patient) of Salmonella typhi are of special significance and of particular concern. Typhoid, caused by this species, is a very serious disease. This case, found in a two-year-old boy who arrived 24 hours earlier from Hong Kong with high fever, represents an imported infection, and should not be considered as suggesting the existence of an endemic reservoir.

The salmonellae are classically identified to the generic level by biochemical means, and definitive identification to antigenic groups and types is performed by serologic examination. The biochemical testing, therefore, is of limited significance.

The Api data base listings for Salmonella only name two taxa (Salmonella sp. and S. typhi) that coincide with this study. Thus, a biochemical comparison of this study to Api becomes even more difficult and insignificant. Nevertheless, one unusual set of results was observed for a

single strain identified as S. typhimurium: ONPG and indole were positive. The Api listings for Salmonella sp. show 1.9% and 3.0%, respectively, for these tests, which are based on 3,154 isolates of various types. However, the Api synonym for Salmonella sp. is S. enteritidis, which is of a different serogroup and therefore not closely related to S. typhimurium. Furthermore, this particular isolate was tested twice with the Api20E, and the two sets of tests yielded different results (profile numbers 5-744-752 and 4-504-752), which suggests the possibility of contamination.

The antimicrobial test results were very similar to those of the shigellae. The salmonellae showed only two strains resistant to ampicillin. These strains are of more interest, however, when considering that one was resistant to five additional drugs, and the other was resistant to four additional drugs. It is very likely that these are examples of the problem of multiple drug resistance that is discussed with great concern in the 1980 article of WHO.

Campylobacter

Since the introduction of media selective for Campylobacter (Dekeyser et. al., 1972), the isolation of those species found in humans has been quite successful. Many workers today find the incidence of C. jejuni to be equal to or greater than that of Salmonella and/or Shigella (Blaser and Reller, 1981; WHO Scientific Working Group, 1980). The findings of this study indicate an incidence of

C. jejuni (61%) far in excess of Shigella (23%) and Salmonella (16%) combined.

Although C. jejuni is the most frequent and serious pathogen of the campylobacters, other closely related species--C. fetus subsp. fetus and C. coli--may also infect humans (King, 1957, 1962; Vèron and Chatelain, 1973; Smibert, 1974, 1978; Blaser and Reller, 1981).

The procedures used at Dameron for identifying Campylobacter as C. jejuni (including only one VPTK plate incubated at 42C) are probably inadequate. These conditions are ideal for C. jejuni and C. coli, but C. fetus subsp. fetus is unable to survive at this temperature. Infections by C. fetus subsp. fetus may therefore be missed. In two instances during this study, curved rods, typical of Campylobacter, were seen in the specimen smear but no Campylobacter species were isolated. One possible explanation is that the specimen contained C. fetus subsp. fetus, which failed to grow at 42C.

Karmali et. al. (1980) state that some variability exists in the temperature-dependent growth of Campylobacter species. Therefore, the authors have utilized naladixic acid (30μg discs) and cephalothin (30μg discs) as added tests to differentiate C. jejuni (no zone for cephalothin, large zone for naladixic acid) from C. fetus subsp. fetus (no zone for naladixic acid, large zone for cephalothin). No mention is made of C. coli. Vèron and Chatelain (1973), however, have also used naladixic acid in differentiating

the above species and have found both C. jejuni and C. coli to be inhibited by naladixic acid. Reliable and simple methods are available to differentiate C. fetus subsp. fetus from C. jejuni and C. coli. No such methods have been presented to differentiate between the latter pair of organisms. C. coli is only a rare inhabitant of humans and so perhaps it is of little significance. Further study of the response of C. coli to other antibiotics might reveal other differences between it and C. jejuni.

Three months ago (January, 1983) a recommendation was made to Dameron Hospital Microbiology Department, during the course of this study, to use two VPTK plates, one incubated at 36C, the other at 42C. Dameron Hospital has complied with this recommendation. The placement of a 30µg disc of naladixic acid on each plate incubated a 42C would contribute to the rapid but presumptive identification of C. jejuni, and has been incorporated into the new procedure. So far, the results have indicated that no strains. of C. jejuni grow at 36C. Reports in the literature (Smibert, 1978; Karmali et. al., 1980; Blaser and Reller, 1981) indicate that C. jejuni grows best at 42C, and is variable or slow-growing at 37C.

The 17 isolates of C. jejuni that were tested by the Api20E and the Api20A proved to be inert biochemically, as expected. One of the original reasons for establishing the genus Campylobacter was the fact that certain "vibrios" were unable to ferment carbohydrates (Sebald and Véron,

1963). More recently, Vèron and Chatelain (1973) reviewed and studied the taxonomy of the genus, employing several biochemical tests. These investigators, however, resorted to seldom-used tests such as growth in the presence of various bactericidal substances. The only test used by Vèron and Chatelain that appears on either of the two Api systems used in this study is the H_2S test. Their results indicate that C. jejuni is weakly positive for H_2S in a "standard" medium, and positive for H_2S in a "sensitive" medium.

The antimicrobial drugs used in the treatment of campylobacteriosis are tetracycline and erythromycin--the former in adults and the latter in children under the age of 12. The present study indicates that all strains of C. jejuni are uniformly susceptible to erythromycin. As to tetracycline, one strain of C. jejuni showed complete resistance, evidenced by no zone whatever; three others had zones of inhibition of 8, 9 and 9mm, probably too small to be considered susceptible. If this in vitro study reflects an in vivo response, then the suggestion is that tetracycline may not be the ideal chemotherapeutic drug, and perhaps erythromycin should be used exclusively.

This study also suggests that the isolation and identification of Campylobacter species may be accomplished by the following tests:

1. direct inoculation of two VPTK media.

2. placing a disc of naladixic acid and a disc of cephalothin on each of the two inoculated plates.

3. incubation of one plate at 42C under reduced O_2 tension and increased CO_2 pressure, the other at 36C under similar physical conditions.

4. perform oxidase test and gram stain on all isolates.

SUMMARY

Of 973 stools and rectal swabs cultured at Dameron Hospital between January 1 and December 31, 1982, 132 (13.8%) were positive. Campylobacter jejuni was responsible for 61% of all infections. Shigella sonnei accounted for 17%, Sh. flexneri 4.5% and Sh. boydii for 1.5% of all infections. In the genus Salmonella, S. typhimurium was recovered from 6.7% and S. enteritidis from 2.2% of infections; S. give, S. heidelberg and S. mbundaka each caused 0.7% of infections; S. newport and S. typhi were each found in 1.5% of all infections. Two isolates of Salmonella were not identified. No Yersinia species were encountered.

Antibiotic and biochemical studies were performed on all Shigella and Salmonella isolates. Biochemically, using the Api20E and the Api20A, C. jejuni was shown to be inert. Antimicrobial testing suggests that not all strains of C. jejuni are susceptible to tetracycline. In contrast, all strains were susceptible to erythromycin.

Appendix 1

Composition of Media

Blood Agar Plate

Tryptose 10g, beef extract 3g, NaCl 5g, agar 15g, distilled water 1000g, 5% defibrinated sheep blood 40ml.

Eosin-Methylene Blue

Peptone 10g, lactose 5g, sucrose 5g, dipotassium phosphate 2g, agar 13.5g, eosin Y 0.4g, methylene blue 0.065g, distilled water 1000g.

Hektoen Enteric

Proteose peptone 12g, bile salts 9g, yeast extract 3g, lactose 12g, salicin 2g, sucrose 12g, NaCl 5g, Sodium thiosulfate 5g, ferric ammonium citrate 1.5g, acid fuchsin 0.1g, bromothymol blue 0.065g, distilled water 1000g.

Salmonella-Shigella

Beef extract 5g, proteose peptone 5g, lactose 10g, bile salts 8.5g, sodium citrate 8.5g, sodium thiosulfate 8.5g, ferric citrate 1g, agar 13.5g, brilliant green 0.00033g, neutral red 0.02g, distilled water 1000g.

Vancomycin-Polymyxin B-Trimethoprim-Kanamycin

Pancreatic digest of casein 15g, papaic digest of soybean 5g, NaCl 5g, agar 15g, 7% lysed defibrinated horse blood 70ml, vancomycin 10mg, polymyxin B 2500IU, trimethoprim lactate 5mg, cephalothin 15mg, distilled water 1000g.

Yersinia Selective Agar
(plus Antimicrobic Supplement CN)

Yeast extract 2g, peptone 17g, proteose peptone 3g, mannitol 20g, sodium deoxycholate 0.5g, sodium cholate 0.5g, NaCl 1g, sodium pyruvate 2g, magnesium sulfate, heptahydrate 10mg, agar 13.5g, neutral red 30mg, crystal violet 1mg, irgasan 4mg, cefsulodin 4mg, novobiocin 2.5mg, distilled water 1000g.

Selenite Broth

Sodium hydrogen selenite 4g, sodium phosphate 10g, peptone 5g, lactose 4g, distilled water 1000g.

Mueller-Hinton Agar

Beef infusion 300g, peptone 17.5g, starch 1.5g, agar 17g, distilled water 1000g.

Mueller-Hinton Chocolate Agar

Mueller-Hinton Agar, 5% defibrinated sheep blood 40ml (added before agar has cooled).

Mueller-Hinton Broth

Beef infusion 300g, peptone 17.5g, starch 1.5g, distilled water 1000g.

Thioglycollate Broth

Peptone 20g, l-cystine 0.25g, glucose 6g, sodium thioglycollate 0.5g, NaCl 2.5g, sodium sulfite 9.1g, distilled water 1000g.

Appendix 2

Biochemical Principles of the Tests on the Api20E

test	biochemical principle
ONPG	Hydrolysis of ONPG by beta-galactosidase.
ADH	Transformation of arginine into ornithine, ammonia and carbon dioxide by arginine dihydrolase.
LDC	Transformation of lysine into cadaverine by lysine decarboxylase.
ODC	Transformation of ornithine into putrescine by ornithine decarboxylase.
CIT	Utilization of citrate as the sole source of carbon.
H_2S	Production of hydrogen sulfide from thiosulfate.
URE	Release of ammonia from urea by urease.
TDA	Formation of indolepyruvic acid from tryptophane by tryptophane deaminase.
IND	Formation of indole from tryptophane.
VP	Production of acetoin from pyruvate during glucose metabolism.
GEL	Liquefaction of gelatin by proteolytic enzymes.
GLU MAN INO SOR RHA SAC MEL AMY ARA	Formation of acid from utilization of carbohydrates.

Appendix 3

Biochemical Principles of the Tests on the Api20A

test	biochemical principle
IND	Formation of indole from tryptophane.
URE	Release of ammonia from urea by urease.
GLU MAN LAC SAC MAL SAL XYL ARA	Formation of acid from utilization of carbohydrates.
GEL	Liquefaction of gelatin by proteolytic enzymes.
ESC	Hydrolysis of esculin.
GLY CEL MNE MLZ RAF SOR RHA TRE	Formation of acid from utilization of carbohydrates.

Appendix 4

Interpretation of Api Profile Numbers

The Api profile number is a numerical representation of the results of the 20 biochemical reactions in the Api kits:

20 test results
+--+-------++--+---+- ➡ profile number
1-104-112

Analytab Products publishes an index that lists the profile numbers and the corresponding identities of the species that can produce such numbers. This is a quick and easy way to identify bacteria:

profile number	identity
1-104-112	*Shigella sonnei*
1-104-152	*Shigella sonnei*
0-004-100	*Shigella flexneri*

Each biochemical test is assigned a numerical value. If a test is positive, then its value is entered. A negative test is "worth" a zero value. After all values have been entered, they are added in groups of three to get the seven-digit "code," or profile number:

	ONPG	ADH		LDC		ODC	CIT	H_2S	URE	TDA	IND	VP	GEL	GLU	MAN	INO	SOR	RHA	SAC	MEL	AMY	ARA	OXI
projected value:	1	2	4	1	2	4	1	2	4	1	2	4	1	2	4	1	2	4	1	2	4	1	2	4
test result:	+	-	-	+	-	-	-	-	-	-	-	+	+	-	-	+	-	-	-	+	-			
entered value:	1	0	0	1	0	0	0	0	0	0	0	4	1	0	0	1	0	0	0	2	0			
addition:	1+0+0			1+0+0			0+0+0			0+0+4			1+0+0			1+0+0			0+2+0				
profile number:	1		-	1			0			4		-	1			1			2				

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